· PutC3 (a)

(b)

(c)

23×

10

providing a plurality of first PCR primers, each first primer having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of interest and a region of randomized nucleotide sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence;

providing a plurality of second PCR primers, each second primer having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence; and then

amplifying the nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers[; whereby] under conditions wherein a subset of the plurality first primers binds to the consensus sequence of interest substantially wherever it occurs in the template, and a subset of the plurality of second primers binds to the template at locations removed from the first primers such that DNA regions flanked by the first primer and the second primer are specifically amplified.

15

12.

(TWICE-AMENDED) A method of amplifying exons from a DNA template comprising:

20 4

(b)

providing a plurality of first PCR primers, each first primer having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of a 3' splice region and a region of randomized nucleotide sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence;
providing a plurality of second PCR primers, each second primer having a

region of fixed nucleotide sequence reversely complementary to a consensus sequence of a 5' splice region and a region of randomized nucleotide sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence; and then

(c) amplifying the DNA template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers[; whereby] under conditions wherein a subset of the plurality first primers binds to a sequence reversely

30

25

Bronk

complementary to the 3' splice consensus sequence substantially wherever it occurs in the template and a subset of the plurality of second primers binds to the 5' splice consensus sequence substantially wherever it occurs in the template, such that exons flanked by the first primer and the second primer are specifically amplified.

5

10 /

(TWICE-AMENDED) A method of amplifying regions flanking a consensus sequence in a nucleic acid template of totally or partially unknown sequence comprising:

(a) providing a plurality of first PCR primers, each first primer having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of interest and a region of randomized nucleotide sequence located 5' to, 3' to, anywhere within, or flanking the region of fixed nucleotide sequence;

(b) providing a plurality of second PCR primers, each second primer having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5 to, 3 to, anywhere within, or flanking the region of fixed nucleotide sequence; then

(c) amplifying the nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers[; whereby] under conditions wherein a subset of the plurality first primers binds to the consensus sequence of interest substantially wherever it occurs in the template, and a subset of the plurality of second primers binds to the template at locations removed from the first primers such that DNA regions flanked by the first primer and the second primer are specifically amplified; then

(d) incorporating the amplified nucleic acid of step (c) into a library;

(e) sequencing a portion of amplified nucleic acid from a particular clone from the library of step (d) and providing a third PCR primer of unique sequence which will prime PCR amplification from the sequenced portion of DNA;

(f) providing a plurality of fourth PCR primers, each fourth primer having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized

25

20

15